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# A potential role of autophagy-mediated vascular senescence in the pathophysiology of HFpEF

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Heart failure with preserved ejection fraction (HFpEF) is one of the most complex and most prevalent cardiometabolic diseases in aging population. Age, obesity, diabetes, and hypertension are the main comorbidities of HFpEF. Microvascular dysfunction and vascular remodeling play a major role in its development. Among the many mechanisms involved in this process, vascular stiffening has been described as one the most prevalent during HFpEF, leading to ventricular-vascular uncoupling and mismatches in aged HFpEF patients. Aged blood vessels display an increased number of senescent endothelial cells (ECs) and vascular smooth muscle cells (VSMCs). This is consistent with the fact that EC and cardiomyocyte cell senescence has been reported during HFpEF. Autophagy plays a major role in VSMCs physiology, regulating phenotypic switch between contractile and synthetic phenotypes. It has also been described that autophagy can regulate arterial stiffening and EC and VSMC senescence. Many studies now support the notion that targeting autophagy would help with the treatment of many cardiovascular and metabolic diseases. In this review, we discuss the mechanisms involved in autophagy-mediated vascular senescence and whether this could be a driver in the development and progression of HFpEF.

KEYWORDS

**autophagy, HFpEF - heart failure with preserved ejection fraction, vascular senescence, vascular aging, obesity, diabetes, hypertension**

## Cardiovascular diseases and HFpEF

Cardiovascular diseases (CVDs) are the leading cause of death worldwide, resulting in 17.9 million deaths in 2019 (1). On top of the list of most prevalent CVDs is heart failure (HF), a progressive condition in which the heart is unable to pump enough blood to the body and provide the required oxygen levels to fulfill its metabolic demands (2). HF represents the end-stage of multiple cardiac injuries linked to cardiovascular diseases and risk factors; therefore, its prevalence has steadily increased during the last decade affecting approximately 1-3% of the total adult population (3). In the USA, HF affects 5.8 million individuals (2.4% of the population) and is the first cause of hospital admission in adult patients, with a readmission rate during the first six months after discharge of 50% (4). According to the ejection fraction (EF), HF can be classified as HF with reduced EF (<40%, HFrEF), HF with mid-range EF (40-50%, HFmrEF) and HF with preserved EF (>50%, HFpEF). Approximately half of the patients with signs and symptoms of HF have HFpEF. Predisposing risk factors for HFpEF include older age, diabetes, obesity, and arterial hypertension (5). Even though these syndromes show similar symptoms (edema, dyspnea, fatigue, exercise intolerance), it is well described in the literature that they are quite different syndromes: HFpEF is characterized by diastolic dysfunction, altered ventricular relaxation and filling, increased stiffness, and concentric remodeling of the ventricular wall, resulting in an important pressure overload. On the other hand, HFrEF is characterized by systolic dysfunction, altered ventricular contraction, which reduces ejection fraction, and an eccentric myocardial remodeling followed by ventricular dilation, resulting in ventricular volume overload (2). Compared to HFrEF, HFpEF presents with increased cardiac perivascular fibrosis, less nitric oxide (NO) bioavailability, earlier endothelial dysfunction and higher level of pro-inflammatory cytokines (6). Additionally, HFpEF patients present a higher load of comorbidities, mainly advanced age, obesity, diabetes and hypertension (7, 8). Dunlay et al., (3) reported a summary of an important number of clinical trials using interventions well described for HFrEF that have shown little or no effect on mortality rates in patients with HFpEF, underlying the importance of the study and development of new therapeutic strategies to treat HFpEF.

HFpEF is associated with a poor quality of life, crucial healthcare resource utilization, high rates of hospitalization, and mortality that are similar to patients with HFrEF (9). Incidences of obesity and diabetes mellitus are projected to grow, leading to an increased prevalence of risk factors for HFpEF (10, 11). HF prevalence of both types increases with age, but the prevalence of HFpEF at any given age increases more rapidly than HFrEF prevalence (12). One of the main limitations of early studies in HFpEF was the lack of established diagnostic criteria (13). In many registries, patients diagnosed with HFpEF also had other comorbidities that could account for their symptoms, such as extreme obesity, lung disease, and myocardial ischemia (13). It was only recently that The European Society of Cardiology proposed a comprehensive set of diagnostic criteria. These criteria allowed for identifying patients with HFpEF, ruling out confounding comorbidities, and reaffirming the existence of the clinical problem (14).

HFpEF is considered a clinical syndrome rather than a discrete disease (9). Therefore, multiple pathophysiological mechanisms, including diastolic dysfunction, are responsible for its generation (9, 15). In recent years, chronic systemic microvascular inflammation has been highlighted as one of the main pathophysiological mechanisms of HFpEF, and the importance of the different comorbidities that induce this response, mainly age, obesity, diabetes, and hypertension, has been emphasized (7, 16, 17). Patients with HFpEF show elevated inflammatory markers such as Interleukin-1 type I receptor (IL-1R), tumor necrosis factor α (TNFα), C-reactive protein (CRP), vascular cell adhesion molecule-1 (VCAM-1) and IL-6 (7, 15). This leads to increased endothelial reactive oxygen species (ROS) production, less NO bioavailability, and nitrosative stress due to the accumulation of nitrogen reactive species (RNS) (7, 18).

In this review, we go through available data regarding vascular and microvascular dysfunction related to autophagy and cell senescence, to propose a potential role of these processes in the development and progression of HFpEF.

## Vascular contribution to HFpEF

### Microvascular dysfunction

The microvasculature is comprised of arterioles, capillaries and venules; microcirculation through these vessels allows the delivery of oxygen and nutrients to meet the energetic demands of local tissues, mainly through regulation of vascular tone, structural microvascular adaptations such as angiogenesis, and the regulation of hemostasis, inflammation and vascular permeability (19, 20). The role of microvascular dysfunction, usually described as an impaired regulation of blood flow in response to oxygen requirements (21), has been widely described in several chronic conditions, such as hypertension, diabetes, obesity, and HF (22). As previously mentioned, Paulus and Tschope (7) proposed a new paradigm for HFpEF in which myocardial dysfunction is, in part, due to coronary microvascular inflammation. They proposed that low-grade chronic systemic inflammation, mainly because of the presence of multiple comorbidities, initiates detrimental microvascular changes that result in the HFpEF-associated myocardial dysfunction. Accordingly, inflammatory markers such as IL-6, CRP and TNFα showed a stronger association in HFpEF patients when compared with HFrEF patients (23). In

summary, it is proposed that inflammation induces ROS production in the endothelium, which decreases NO bioavailability and increases peroxynitrite levels, impairing the guanylate cyclase/cyclic guanylate monophosphate (cGMP)/protein kinase G (PKG) pathway in cardiomyocytes (24). This was further described in cardiomyocytes of a mice model of HFpEF treated with high fat diet (HFD) and N(ω)-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase (NOS) inhibitor (18). Whether the same effects occur in VSMCs is yet to be determined. Additionally, HFpEF patients show systemic peripheral impaired microvascular reactivity evaluated by endoPAT, a non-invasive endothelial dysfunction test (25), and capillary rarefaction in skeletal muscle associated with poor exercise tolerance (26). Moreover, a decrease in coronary microvascular function, an increased prevalence in coronary rarefaction, and an impaired maximal hyperemia, compared to age-matched controls, were also reported in HFpEF patients (25–28). For a more comprehensive review on this subject, see Weerts et al., (29).

Recently, using cell therapy, de Couto et al., (30) administered intracoronary cardiosphere-derived cells (CDCs) during two weeks to HFpEF rats, developed by feeding Dahl salt-sensitive rats with a high salt diet. CDCs treatment improved EC-dependent vasodilation, reduced oxidative stress, restored endothelial NOS (eNOS) expression, previously shown to be decreased in the HFpEF endothelium (31), inflammatory response and VCAM-1 expression. It also improved diastolic dysfunction and restored vascular reactivity (30). These results uncover the importance of microvascular dysfunction in HFpEF. Another approach to evaluate microvascular function is the study of the retinal arterioles, which can be evaluated using non-invasive optic cameras (32). Retinal arterioles wall-to-lumen ratio (rWLR) was significantly higher in HFpEF group, consistent with a previously proposed correlation between retinal arteriole structural alterations and HF (33). Interestingly, this increase was also significant when compared to hypertensive controls (32). Finally, a recent study performed by Yuksel et al., (34), evaluated microvasculature using nailfold videocapillaroscopy in HFpEF patients and found abnormal results regarding microvascular morphology, architecture and density compared not also with control patients but also with patients with HFrEF.

Taken together, the evidence presented above strengthens the previously proposed role of microvascular dysfunction in HFpEF pathogenesis (Figure 1). However, remains to be elucidated whether this microvascular dysfunction is cause or consequence of HFpEF.

## Macrovascular dysfunction

Arterial intima-media thickness and stiffness have been identified as important risk factors for HF (35). The assessment of major vessel function in HFpEF started with aorta analyses from HFpEF patients. These aortas present a diminished aortic distensibility (36), a decreased vasorelaxation response induced by nitroglycerine (37) and exercise (38, 39), increased resting vasoconstriction and less NO sensibility (17). Furthermore, an increased aortic stiffness has been widely reported in these patients (17, 40–42). Schwartzenberg et al., (43) reported that HFpEF patients responded better to treatment with sodium nitroprusside (a vasodilator drug), as compared with HFrEF patients, underlying the importance of the vascular component to HFpEF. Among the mechanisms that relate arterial stiffening to myocardial dysfunctions are an impaired myocardial oxygen supply, reduced arterial compliance and a decrease in diastolic blood pressure, that compromise coronary artery flow (44).

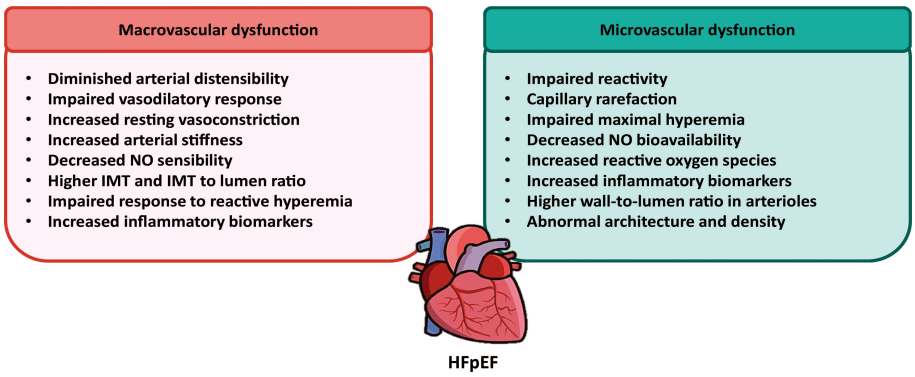


FIGURE 1
Contribution of macrovascular and microvascular dysfunctions to the development of HFpEF. Using non-invasive techniques, several macro and microvascular alterations have been described in the resistance and conduit arteries from HFpEF patients. These alterations are suggestive for a main role of vascular dysfunction in the pathogenesis of HFpEF.

An association between HFpEF carotid arteries morphological and functional alterations has been recently described (32, 45, 46). Using ultrasound and magnetic resonance imaging (MRI) on patients from the multi-Ethnic study of atherosclerosis (MESA), Fernandes et al., (44) found a relation between carotid distensibility and enhanced left ventricle diastolic function, even after adjustment for risk factors and blood pressure control therapies. In another study that analyzed MESA patients, no difference in the association of carotid intima-media thickness (IMT) was reported when comparing HFpEF with HFrEF patients (46). Additionally, after adjustment with traditional risk factors, the observed association of internal and common carotid IMT and HF lost its statistical significance. Moreover, it was recently described that common carotid arteries of HFpEF patients had a higher diameter IMT and a significantly higher IMT to lumen ratio compared to controls (32). Interestingly, blood pressure control decreases carotid IMT but did not stop its progression in hypertensive subjects (47). Engstrom et al., (48), reported that there was a significant association of carotid IMT and HF hospitalization. Through the evaluation of brachial-ankle pulse wave velocity (baPWV), a method to estimate arterial stiffness, which reflects the stiffness of both the aorta and peripheral artery, Hu et al., (49), described a positive correlation between baPWV and left ventricular hypertrophy and diastolic function. Similarly, using brachial artery flow-mediated dilation (FMD) and microvascular function *via* reactive hyperemia (RH), it was reported that HFpEF patients had a reduction in brachial artery diameter in response to RH, compared to aged-matched controls (16). Nevertheless, this difference was lost when normalizing for shear stress rate differences. In accordance with the latter, no differences were found between HFpEF patients' femoral artery FMD and those of the controls (50). On the other hand, a totally different result was described by Farrero et al., (51), in which a significant reduction in brachial artery FMD was observed in HFpEF patients compared with hypertensive controls. To further support the importance of the vasculature to HFpEF pathophysiology, several biomarkers related with vascular function are altered during HFpEF: reduced NO bioavailability (potent vasodilator), higher levels of endotelin-1 (a potent vasoconstrictor) and increased levels of plasminogen activator inhibitor-1 (PAI-1), a described risk factor for atherosclerosis, among others (52).

It is important to note that most of the studies discussed in this section have been performed using non-invasive methods, such as MRI, FMD, and ultrasound. Additionally, human studies are usually performed with patients taking medication for HFpEF comorbidities, mainly anti-hypertensive drugs, which limits the data obtained about the natural history of the disease. Very little research focusing on molecular alterations have been performed, especially in relation to macrovascular circulation. This may be due to the, until recently, lack of animal models that properly emulate HFpEF characteristics. In the past few years, an important number of animal models have been developed, so further investigation will hopefully reveal more detailed information about vascular dysfunction and remodeling during HFpEF onset and progression. Even though there is a long way to go to fully elucidate the molecular mechanisms behind vascular alterations in HFpEF, it is now clear that micro and macrovascular dysfunctions could play a major role in HFpEF (Figure 1).

## Vascular aging and senescence

The mechanisms of aging and age-associated disorders are complex and involve cellular senescence (53). Aging is characterized by an accumulation of senescent cells, as a result of aged organisms being unable to repair damage at the same rate as cells become damaged (54). Senescent cells can be identified by a permanent cell growth arrest (55, 56). Senescent cells become enlarged and flattened, with proliferative arrest, that secretes pro-inflammatory molecules, a phenotype known as senescence-associated secretory phenotype (SASP), triggering chronic sterile inflammation that induces tissue remodeling (53). Several markers are used to indirectly detect senescent cells, being senescence-associated β-galactosidase (SA β-gal) activity the most common. Lysosomal β-gal activity is detected at a low pH (around pH 4) but becomes detectable at a higher pH (pH 6) in senescent cells due to marked expansion of the lysosomal compartment (57). Other markers of cellular senescence include high expression of p53, p16, p21, p38-mitogen activated protein kinase (MAPK), and phosphorylated histone H2AX (γH2AX), an early marker of cellular response to the induction of DNA double-strand breaks (58–62). Moreover, high mobility group A (HMGA) proteins and heterochromatin markers, including heterochromatin protein-1 and tri-methylated lysine 9 histone H3, are molecular markers of senescence-associated heterochromatin foci and are considered to reveal cellular senescence (61).

Arterial remodeling occurs with aging, even in the absence of cardiovascular disease and cardiovascular risk factors. Aging is frequently associated with vascular dysfunction (63, 64). In fact, people with progeria syndrome, that present a premature aging in early childhood, developed premature atherosclerosis disease (65). Aged arteries have increased intima/media thickness ratio, with an increase of 2- to 3-fold from 20 to 90 years of age (66, 67). The arterial media also becomes thicker with aging, and its cellularity decreases simultaneously (68). Furthermore, the length and circumference of the aorta increase with aging (69), with an accumulation of collagen and elastin decline (70). These structural changes are associated with a reduction in compliance, reduction of elasticity/distensibility, and increase of stiffness, resulting in higher systolic blood pressure and lower diastolic pressure (71). ROS and chronic low-grade sterile inflammation are two significant contributors to the progression of age-related

vascular dysfunction. Senescent cells accumulate in the arteries with aging irrespective of whether a person has or not age-related vascular disorders (72–75). Along with aging, vascular tissues of rodents and humans show elevation of the levels of p16, p21, phosphorylated p38-MAPK, and double-stranded DNA breaks, in association with high SA β-gal activity (76–79). Expression of p53 and p21 is increased in the arteries of elderly persons, together with a structural breakdown of telomeres (75). Interestingly, senescent cells are increased in the coronary arteries of patients with ischemic heart disease but not in the internal mammary arteries (72).

Blood vessel walls are comprised primarily of ECs, VSMCs and extracellular matrix (ECM). Because in both cells are described the occurrence of phenotypic features commonly observed in senescent cells (74), and changes in vascular ECM structure is associated with aging (80), the association of these 3 vascular components with senescence and CVDs is next described.

## Vascular smooth muscle cells

VSMCs are the key component of the medial layer in arteries, with an important role in contraction and regulation of blood pressure and vascular tone (81). Aging-dependent functional changes of VSMCs are partly due to deregulation of TGF-β signaling, and these cells undergo a transformation from "contractile" to "synthetic" phenotype (82, 83). The VSMC synthetic phenotype is responsible for the aging-dependent intimal thickening because of the increased proliferation, migration and production of collagen (70). Moreover, upon metabolic alterations, the shift from a contractile to a synthetic phenotype has been associated with progression of hypertension and atherosclerosis (84).

Intimal thickening is also associated with the formation of atherosclerotic lesions (85). Interestingly, senescent VSMC have been identified in atherosclerotic lesions of patients with coronary artery disease and peripheral artery disease (72). Those VSMC present shorter telomeres, are positive for SA β-gal, and have elevated p16 and p21 expression (74, 86). Senescent VSMC in atherosclerotic plaque display loss of telomeric repeat-binding factor-2 (TRF2). TRF2 overexpression reduces DNA damage, accelerates DNA repair, and suppresses cellular senescence (87). VSMC specific knockout (KO) of TRF2 increases atherosclerosis and necrotic core formation. These pathological changes are inhibited in mice with VSMC-specific overexpression of TRF2 (87). Hypertension, an established risk factor for HFpEF, increases the activity of p53 and p21 in the arteries of hypertensive patients. While telomere length is comparable between patients with hypertension and controls, telomere uncapping is 2-fold higher in hypertensive patients (88). A murine model of genomic instability showed senescence of ECs and VSMC in the aorta, along with impaired vasodilation, increased vascular stiffness, and hypertension (89). In a hypertensive rat model, produced by treating with deoxycorticosterone acetate and salt, overexpression of p16 was detected in the coronary arteries, indicating the existence of a vicious circle between cellular senescence and hypertension (90). Another vicious circle is produced because senescent VSMCs trigger low grade sterile inflammation through the secretion in the SASP of several cytokines, including IL-1α (91). Moreover, SA β-gal positive VSMC in carotid plaques express IL-6, suggesting that senescent VSMCs have a SASP involved in the progression of atherosclerotic disorders (91). Taken together, these studies show that senescent cells accumulate in the vessels of patients with atherosclerosis, hypertension, aneurysms, and intimal hyperplasia, some of them common risk factors for the development of HFpEF.

ROS and angiotensin (Ang) II are well-known inducers of senescence in VSMC (92, 93). Ang II administration also induces senescence of VSMCs in apolipoprotein E null mice (94). Ang II promotes VSMC senescence by suppressing Mdm-2-mediated degradation of p53 and promoting the expression of smooth muscle 22α (SM22α) (95). Similarly, we described that Ang II increases contractile proteins calponin and α-smooth muscle actin (α-SMA) (96). ROS induces DNA damage in VSMC and suppresses telomerase activity, leading to telomere shortening and cellular senescence in the atherosclerotic lesion (86). On the other hand, hypoxia inhibits senescence by promoting telomerase activity (97).

As reviewed by Chi et al., (98), epigenetics can accelerate or prevent VSMC senescence. Autophagy, the mechanism by which cells removes damaged components (99) could also prevent VSMC senescence as vascular aging is associated with impaired autophagy (100) and induced moderate autophagy can increase proliferation in VSMCs (101). Although it is possible to detect senescence in VSMCs using common senescence criteria, such as changes in levels of p16, p21, p38-MAPK, p53 and H2A.X and SA β-gal activity (102), in a study aimed to characterize human coronary VSMC senescence it was concluded that classical senescence markers show a mild deregulation as to warrant consistent senescence detection *in vitro*, and that altered RNA metabolism could be a key feature to ensure VSMC senescence detection (103).

## Endothelial cells

The endothelium is a semipermeable barrier composed by a monolayer of cells that controls the exchange of nutrients and metabolites, regulates vascular tone, permeability, inflammation and blood fluidity and, thereby, is of paramount importance for maintaining vascular homeostasis (104, 105). ECs are a constant target for different damage-inducing factors present in the bloodstream that may impair cellular function. EC senescence can occur as a result of this cell damage, thereby precluding

uninhibited proliferation of damaged cells, but this process can also be harmful and contribute to the pathophysiology of cardiovascular diseases (106). During aging, ECs display the classic markers of cell senescence (64). Additionally, alterations in mitochondrial biogenesis (107), NFkB activation (108), increased matrix metalloproteinase (MMP) secretion (109) and reduced eNOS activity (110) have also been described. In human umbilical vein ECs (HUVECs), knockdown of the transcription factor E2F2 induced senescence in these cells and its overexpression decreased senescence markers; interestingly, a lower expression of E2F2 was seen in aortas of aged mice (111). These experiments suggest that E2F2 can be a potential target to modulate senescence *in vivo*, yet its participation in HFpEF remains unknown. Finally, during aging, Sirtuins dysregulation occurs in the endothelium, particularly a decrease in SIRT1, which induces cell senescence and has been linked to the development of CVDs, as reviewed by Kida and Goligorsky (112). Nevertheless, Conti et al., (113) found no significance differences in SIRT1 levels in peripheral blood mononuclear cells of HFpEF patients compared to controls. Whether these differences are due to the different cell type studied or not requires to be studied.

## Vascular extracellular matrix

The ECM is composed of several structural proteins, including elastin and collagens, that not only provides structural support to the VSMCs and ECs, but also regulates the mechanical function of the vessel (80). Arteries stiffen with age, suggesting that age-related arterial stiffening may contribute to CVDs (114). In fact, elastin fibers lose functionality with age mainly by fragmentation, calcification and MMP degradation. These changes induce the formation of stiffer fibrils, which directly contributes to age-dependent increases in arterial stiffness (114). In contrast, the arterial collagen content and collagen crosslinking increases with age (80). Increased fibrosis has been described in the intima (115), media (116, 117), and adventitia (118).

Age-related changes in elastin and collagen composition and function are due to the action of MMPs (80). MMPs and its tissue inhibitors (TIMPs) changed as a function of age in the absence of clinically significant CVD (119). In the blood vessels, age-related MMP-2 upregulation occurs in the human aorta but not in the internal mammary artery (120), and this upregulation is associated to arterial stiffness (121). Moreover, MMP-3 polymorphisms have been associated with vascular remodeling and age-related arterial stiffening (122). Several factors that are dysregulated during vascular senescence, such as NO, IL-1 and TNFα, trigger MMPs synthesis and activation (80). Moreover, MMP activation, which disrupts arterial integrity, can be induced by oxidative stress (123). An interesting study showed a ROS-dependent activation of MMPs in cerebral arteries of aged, but not young, hypertensive mice (109). This finding supports the notion that multiple comorbidities, i.e. age and hypertension, are required for some of the pathological alterations observed in these arteries.

ECM alterations create a pro-inflammatory environment that induce phenotypic alterations in both ECs and VSMCs, such as those observed during vascular aging and senescence (124). Since low-grade chronic inflammation has been proposed as one of the key features of HFpEF, it could be assumed that these ECM alterations play an important role in this disease. Although no studies have been performed that evaluate vascular MMP levels and activity in HFpEF, a cardiome-directed network analysis performed in a rat model of HFpEF showed that ECM alterations occur in the heart of these animals (125). Additionally, a correlation between MMP-2 levels and left ventricle EF was found in HF patients (126). In fact, MMP-2 has been proposed as a target for HF treatment (127). Furthermore, human primary fibroblast from patients with both hypertension and HFpEF showed a significant decrease in membrane type 1-MMP, compared with hypertensive only and healthy patients (128). Whether similar alterations occur in vascular tissue remains to be determined.

# Autophagy, cell senescence and vascular aging

Autophagy is a physiological process that seeks to maintain cellular homeostasis by controlling the degradation of components such as proteins and damaged organelles (129). ECs and VSMCs are no strangers to this process, and multiple diseases have been associated with an imbalance in the autophagic flux (130). Among the most described autophagy hallmarks to evaluate this process are the accumulation of p62, LC3-II levels, LC3-II/LC3-I ratio, the analysis of autophagy related protein (Atg) levels and some of the main regulatory proteins such as Beclin-1, ULK1 and mTOR (131). The relationship between senescence and autophagy has been explored in both VSMCs and ECs.

## Vascular smooth muscle cells

An increase in autophagy has been related to phenotypic changes in VSCMs from a differentiated to a dedifferentiated one, which favors the appearance of different CVDs (130). On the other hand, autophagic flux blockage has also been related to a phenotype change in diseases such as aneurysms and atherosclerosis (132). A direct link between VSMC autophagy and arterial stiffness is demonstrated using a VSMC-specific Atg7-KO mice (Atg7F/F SM22α-Cre+ mice) (133). Moreover, specific deletion of Atg7 in VSMC induces p62 accumulation and accelerates the development of stress-induced premature senescence (134). During aging, an impaired autophagy is triggered due to direct oxidation of Atg3 and Atg7 that inhibits

LC3 lipidation (135) and due to mTOR activation (136). Also, an increase of IL-6 and impairment of mitochondrial function within the aorta, associated with enhanced mitophagy and increased PARKIN levels are observed (137). Furthermore, during aging, the expression of Krüppel-like family of transcription factor 4 (KLF4) decreases in vascular tissues in C. elegans, mice and humans (138). Overexpression of KLF4 increases autophagy flux and improves vessel function in aged mice, suggesting an evolutionary transcriptional regulation of autophagy during aging (138). Accordingly, activation of autophagy by the upregulation of the peroxisome proliferator activated receptor gamma coactivator 1 alpha (PPARGC1A) (139), celastrol (a quinone methide triterpenoid isolated from the Celastraceae family) (140), genistein (141) or nifedipine (142) suppresses VSMC senescence by upregulating autophagic flux.

In both replicative and stimulus-induced *in vitro* senescence models, it has been demonstrated that autophagy is required for VSMC senescence development. In rat VSMC treated with Ang II, an increase in SA β-gal activity, p16, p21, and p53 levels are observed (143, 144). Treatment with Ang II also decreased VSMC proliferation (140). Interestingly, these studies show a decrease in autophagic flux, so using an autophagy inducer such as rapamycin, Ang II-induced senescence is prevented (140, 143). Doxorubicin was also shown to induce VSMC senescence through an autophagy-dependent mechanism. A decreased autophagic flux through activation of mTOR and downregulation of essential autophagy proteins such as Beclin-1 and LC3 were described in these cells (136, 141). Other VSMC senescence inducers have been shown to have a slightly different mechanism. For example, hydrogen peroxide has been shown to induce senescence by blocking autophagic flux, causing LC3 accumulation (142). On the other hand, oxLDL induces senescence but does not produce modification of LC3 levels or ULK1/mTOR phosphorylation. Despite this, rapamycin prevents oxLDL-induced VSMC senescence (101). This proves that even when autophagy is not involved directly in the induction of cell senescence, it can be a rescue mechanism.

In both human and rat VSMC replicative senescence models, increased mTOR signaling is observed, but upon treatment of these cells with rapamycin, all senescence markers are decreased (145). In aged cells, a decrease in Beclin-1 and LC3 levels and an increase in mTOR phosphorylation are also observed. Remarkably, upon increasing autophagic flux, senescence is reversed (146). Finally, Grootaert et al., (134) showed that Atg7-KO VSMC displayed lower proliferation rate and increased senescence markers, consistent with an acceleration of senescence. Although there are some differences in the molecular mechanisms of the different treatments and the time of treatment to induce senescence, the same pattern is observed in all of them; autophagy inhibition is an essential step in the induction of senescence in VSMCs. Therefore, current evidence supports the idea that autophagy activation in VSMC could have protective effects in the aging-associated development of CVDs.

## Endothelial cells

In general terms, autophagy is crucial to maintain the homeostasis of ECs, while impaired autophagic flux can lead to endothelial inflammation and thereby, favor the development of atherosclerosis (147). Recent studies have delved into the role of endothelial autophagy in CVDs. Gogiraju et al., (148) reported that mice lacking the endothelial leptin receptor and subjected to transverse aortic constriction showed improved left ventricular function and reduced hypertrophy. Moreover, deletion of the leptin receptor was associated with increased autophagy and impairment of the Akt/mTOR pathway, suggesting a protective role for autophagy in a pressure overload setting, which is suppressed by leptin signaling (148). Another study reported that mice with EC-specific deletion of autophagy-related protein 7 (Atg7) show increased susceptibility of doxorubicin-induced cardiotoxicity (149). These data further support a protective role for autophagy in CVDs.

The link between endothelial autophagy and senescence has also been explored. Rhynchophylline has been found to reduce Ang II-induced senescence *via* AMPK-dependent activation of autophagy in endothelial progenitor cells (150). In addition, C1q/tumor necrosis factor-related protein 9 (CTRP9), which wields anti-aging and anti-atherogenic effects, was recently found to reduce endothelial senescence induced by palmitic acid in HUVECs, an effect also achieved through AMPK-mediated activation of autophagy (151). Pan et al., (152) showed that the overexpression of Yes-associated protein (YAP) in HUVECs and in rat aortas increased the activity of SA β-gal staining and protein markers such as p16, p21 and p53, along with an activation of mTOR pathway and a blockage of autophagic flux. They also demonstrated that the knockdown of YAP and the inhibition of mTOR could relieve both cellular and vascular senescence (152). Advanced oxidation protein products (AOPPs) result from cell oxidative stress and are accumulated and increased in patients with vascular disease and aging (153). In HUVECs, AOPPs induced senescence, increasing the expression of p21, p16 and SA β-gal activity, along with an impairment in autophagic flux (154). The effects of AOPPs were also evaluated in a model of $ApoE^{-/-}$ mice fed with a HFD, which showed an increase in senescence molecular markers in aortic tissue (154). Interestingly, it has been reported that autophagy reduces apoptosis and senescence induced by high glucose concentrations in human coronary artery ECs (155). Nonetheless, a protective role for endothelial autophagy and

its potential effect in cellular senescence in the context of HFpEF remains to be elucidated.

## Autophagy and HFpEF

The role of autophagy is well described in HFrEF (156). Although its role in HFpEF is poorly described, there is evidence that autophagy could play an important role in the development of HFpEF. cDNA analysis in a rat model showed that five processes are mainly involved in the development of HFpEF: endothelial function, inflammation, sarcomere/cytoskeleton, extracellular matrix and apoptosis/autophagy (125). RNAseq analysis performed in patients with HFpEF also revealed that genes related to endoplasmic reticulum stress, angiogenesis, and autophagy, are related to the development of HFpEF (157). Similarly, in a model of aged HFpEF mice, through a RNAseq analysis, it was found that the most upregulated pathways were those related to cell cycle and mitotic cell cycle processes in the heart of aged mice (158). Animal studies show that both autophagy and mitophagy decrease in the heart with age (159, 160). LC3-II expression and the LC3-II/LC3-I ratio decrease in the heart of aged mice with diastolic dysfunction, compared to young controls (159). On the other hand, myeloid differentiation protein 1 (MD1) is decreased in the heart of HFpEF mice and its down regulation promotes autophagy through a ROS/MAPK pathway (161). Mitochondrial dysfunction is one of the central mechanisms in the development and progression of HF (162). In this line, mitophagy was found to be decreased in aged mice. Interestingly, p53 was found to inhibit PARKIN translocation into the mitochondria, decreasing mitophagy and thus contributing to mitochondrial dysfunction. In addition, p53-KO mice improved mitochondrial integrity and cardiac functional reserve in both aged and doxorubicin-treated mice. PARKIN overexpression also improved cardiac function and decreased SA β-gal activity (160). These data highlight the importance of autophagy to be recognized as a target to be studied in development and treatment of HFpEF.

## Senescence and HFpEF

EC senescence has been described to play an important role in aging mice with HF (163). The presence of comorbidities associated with age such as hypertension, diabetes or obesity contribute to endothelial inflammation and the consequent reduction of its ability to induce vasodilation, which in turn elicits cardiac hypertrophy, stiffness and ultimately, HF (7, 106). Chronic sterile inflammation, probably due to a SASP, is present in the myocardium of HF patients (164), and is involved in the induction of cardiac remodeling (165). In a murine model of left ventricular pressure overload, cardiac and endothelial p53 levels are increased, leading to cardiac inflammation associated with suppression of myocardial angiogenesis, tissue hypoxia, and cardiac dysfunction (166, 167). These studies suggest the involvement of cardiac senescence in the pathophysiology of HF.

One of the major risk factors for HFpEF is age (5, 9). Moreover, it was recently suggested that EC senescence also contributes to the development of HFpEF: when mice with accelerated senescence were fed a high-fat, high-salt diet, both EC senescence and inflammation increased, along with the typical hemodynamic and structural changes of HFpEF and an impaired endothelial-dependent vasodilation of the aorta (163). Furthermore, the histological analysis of thoracic aorta revealed that the pro-inflammatory protein ICAM-1 and the senescence marker acetyl-p53 were increased in ECs of senescence accelerated mice (SAM) fed with Western diet, as compared with the SAM fed with control diet, suggesting that the potential therapeutic targeting of EC senescence may be a valuable strategy for the treatment of HFpEF (163). Using an aged mice model of diastolic dysfunction, Shinmura et al., (159), showed increased levels of SA β-gal of aged mice. An interesting model of telomerase RNA KO plus diet-induced HFpEF (HFD and L-NAME supplemented-water), showed increased p53 expression in the heart, associated with impaired mitochondrial respiration, and that myocardial-specific p53 KO mice show a delay in the development of HFpEF, although the pathology still developed (168). Patients from the multicenter PROMIS-HFpEF study with a pan-inflammatory phenotype had increased levels of insulin-like growth factor-binding protein 7 (IGFBP7), a protein that stimulates inflammation and cell senescence (169). Accordingly, HFpEF patients from the RELAX trial showed a higher baseline IGFBP7 that was correlated with impaired diastolic function (170). Nevertheless, while the use of senolytics is a promising therapeutic approach (171), the evidence linking senescence to HFpEF is still scarce and more studies are required to confirm these findings.

Considering that cellular senescence induces vascular dysfunction and inflammation, it seems reasonable that it would also promote pathologic changes observed in HFpEF. Moreover, as stated above, chronic microvascular inflammation, a senescence-like phenotype, is one of the hallmarks of HFpEF development (7).

## A possible role of vascular autophagy-mediated senescence in HFpEF

So far, we have presented evidence that: 1) microvascular and macrovascular dysfunction are present in HFpEF and play an important role in its pathophysiology, 2) aging, one of the most prevalent comorbidities of HFpEF involves both vascular senescence and vascular autophagy impairment, 3) autophagy modulates cells senescence in both VSMC and ECs and 4) both autophagy and cell senescence in the heart muscle and

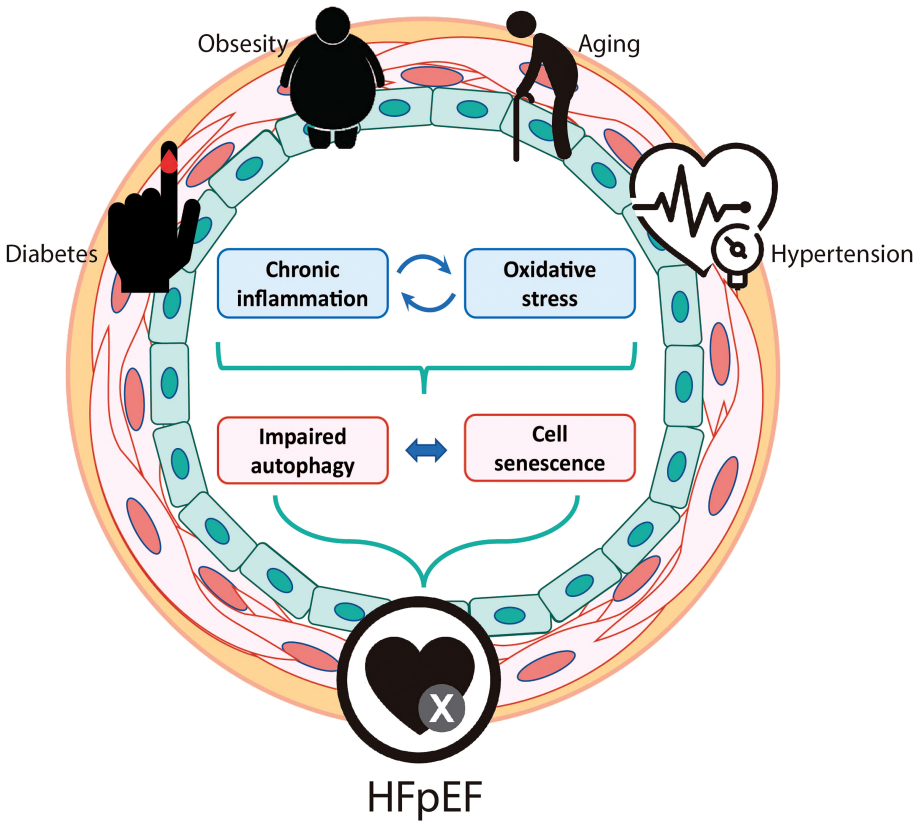


FIGURE 2
Potential role for vascular autophagy and senescence in the development and progression of HFpEF. Predisposing risk factors for HFpEF include older age, diabetes, obesity, and arterial hypertension. All these conditions trigger chronic inflammation and oxidative stress that could impair autophagy and induce cell senescence in both vascular smooth muscle cells and endothelial cells. Vascular senescence could be the responsible for the micro and macrovascular dysfunctions that are described in HFpEF patients and animal models.

endothelium are most likely to be participating in the development and progression of HFpEF.

It is currently believed that chronic low-grade inflammation is one of the main drivers of HFpEF, resulting in a decreased NO bioavailability, an increase in proinflammatory cytokine levels and oxidative stress (7). ROS and inflammation have also been described as characteristic features of vascular aging, and the resulting vascular remodeling and dysfunction (172). It has been shown that ROS induces senescence in both VSMCs and ECs (92, 93, 154), and that this leads to unpaired vasodilation and vascular stiffness, both of which have been observed in HFpEF patients (17, 37–42). As with aging, during hypertension, another important comorbidity of HFpEF, vascular senescence is observed (88). Taken together, this data supports the idea that vascular senescence could be an important component of HFpEF physiopathology. IGFBP7, a protein involved in the regulation of cell senescence, was found to be increased in HFpEF patients (169, 170). So far, only one study has been performed that evaluates senescence markers in a diastolic dysfunction mouse model and shows that there is an increase in p53 levels and SA β-gal activity in aortic ECs of these mice (163).

The inhibition of autophagy leads to cell senescence in both VSMCs and ECs (134, 149). On the same line, autophagy induction can prevent the appearance of cell senescence (101). It has also been described that vascular aging is accompanied by an impaired autophagy, which results in vascular stiffness (135). Oxidative stress, mentioned as an important component of HFpEF, induces VSMC senescence through blockage of autophagy (142). Since autophagy is starting to appear as an emerging pathway involved in HFpEF, mainly through genetic analyses, its relationship with vascular senescence is a promising field of study. Hearts of aged mice with diastolic dysfunction show impaired autophagy (159). Nevertheless, no studies have been performed that evaluate autophagy in vascular tissue during HFpEF, nor its relationship with cell senescence.

## Concluding remarks

In this review, we propose a potential role for vascular autophagy and senescence in the development and progression of HFpEF, focusing on both VSMCs and ECs. Diabetes, obesity, aging and hypertension, main risk factors for HFpEF, triggers chronic inflammation and ROS, that impairs autophagy and triggers cell senescence. The presented data support the idea that both autophagy and cell senescence, processes that are strongly related to one another, might be important components of HFpEF pathophysiology (Figure 2). As mentioned, HFpEF is an increasing healthcare burden worldwide, with no effective treatment. So, it is highly important to open new fields of research that could lead to a better understanding of this disease and the development of new therapeutic targets for its treatment.

## Author contributions

The authors confirm contribution to the paper as follows: review conception and design: FS-O, MT, FP, IN-S, and MC; critical analysis of the literature: FS-O, MT, FP, JM-B, IN-S, JR, MV, and MC; draft manuscript preparation: FS-O, MT, FP, JM-B, IN-S, JR, MV, SL, PC, and MC; SL and MC reviewed the final version of the manuscript. All authors approved the final version of the manuscript.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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